DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for 90-Day Inhalation Toxicity (OPPTS 870.3465)

MRID 47185137

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for 90-Day Inhalation Toxicity (OPPTS 870.3465)
MRID NO:	47185137
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-47
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. 90-Day Inhalation Toxicity.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is not sufficient to support the requested waiver for 90-day inhalation toxicity testing.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for 90-Day Inhalation Testing (OPPTS 870.3465).

Registrant's Justification

In an acute inhalation toxicity study in rats (MRID 47185121), the LC_{50} for Homobrassinolide Technical was 2.26 mg/L (Toxicity Category IV).

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. The metabolism of Homobrassinolide Technical via inhalation is not expected to be different from that resulting from ingestion. It is therefore unlikely that inhalation of the product will result in toxic metabolites.

Reviewer's Conclusion

The ORNL reviewer for the 90-day oral toxicity study found it to be unacceptable. Based on that conclusion, the information submitted is not sufficient to support the requested waiver for 90-day inhalation testing. If the Agency judges the 90-day oral toxicity study to be acceptable, then sufficient information has been submitted to support the waiver for 90-day inhalation testing.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Teratogenicity (OPPTS 870.3700)

MRID 47185138

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
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Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: FEB 21 2008

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Teratogeniciy (OPPTS 870.3700)
MRID NO:	47185138
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-48
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Teratogenicity.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is not sufficient to support the requested waiver for teratogenicity.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Teratogenicity (OPPTS 870.3700).

Registrant's Justification

Homobrassinolide in end use products will be applied to crops in very low amounts (parts per million levels). Its solubility in distilled water is 3.18%, and its other physical and chemical properties suggest it possesses no toxic potential to the environment. Under field conditions, homobrassinolide has been found to be rapidly metabolized in plant tissue, and any residues are rapidly degraded or metabolized. There is no reasonable expectation that pregnant humans or animals would be exposed to homobrassinolide at levels that would induce either maternal toxicity or teratogenicity.

In acute studies, Homobrassinolide Technical was virtually non-toxic (Table 1).

Table 1. Acute toxicity of Homobrassinolide Technical

Oral LD_{50} (rat)	MRID 47185118	>5000 mg/kg	Toxicity Category IV
Oral LD_{50} (mouse)	MRID 47208903	>5000 mg/kg	Toxicity Category IV
Dermal LD_{50} (rat)	MRID 47185120	>2000 mg/kg	Toxicity Category IV*
Inhalation LC_{50} (rat)	MRID 47185121	2.26 mg/L	Toxicity Category IV
Eye irritation (rabbit)	MRID 47185122	Mild irritant	Toxicity Category III
Skin irritation (rabbit)	MRID 47185123	Not an irritant	Toxicity Category IV
Skin sensitization (guinea pig)	MRID 47185124	Not a sensitizer**	--

*Registrant's classification. ORNL reviewer notes this should be Toxicity Category III

**ORNL reviewer classified this study as unacceptable, but upgradable upon submission of an acceptable positive control study

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. Applying a safety factor of 10 gives an estimated NOAEL of 100 mg/kg/day for a two-year chronic toxicity in rodents. To extrapolate the chronic toxicity NOAEL value of 100 mg/kg/day in rodents to a lifetime exposure value for humans, the 100 mg/kg/day value is multiplied by 60 kg (avg body weight for humans), which would give 6000 mg/day. Dividing the 6000 mg/day value by a safety factor of 10 gives an estimated NOAEL for homobrassinolide exposure in human adults of 600 mg/day. This would be the maximum tolerated dose for human adults. Dividing the adult maximum tolerated dose by a safety factor of 10 gives a maximum tolerated dose of 60 mg/day for special groups, including children.

The 90-day oral study in rats showed no toxicity, no changes in blood chemistry, and no gross or histopathologic lesions. There were no maternal toxicity effects that could lead to teratogenic effects.

The following genetic assays using Homobrassinolide Technical were negative: *Salmonella typhimurium* bacterial mutation (4 standard strains tested up to 0.5 µg/plate, with and without metabolic activation) (MRID 47208904); mammalian (mice) chromosomal mutation (up to 2000 mg/kg) (MRID 47208905); and mammalian (mice) DNA damage (up to 2000 mg/kg) (MRID 47185127).

Homobrassinolide Technical showed minimal toxicity to aquatic organisms. In freshwater bioassays with *Poecilis reticulata*, and *Brachydanio rerio* the 96-hr LC_{50} for Homobrassinolide Technical was 24.56 mg/L and 14.38 mg/L, respectively (MRID 47185129). The 48-hr LC_{50} in *Daphnia magna* was 8.90 mg/L (MRID 47185130).

Homobrassinolide and other brassinosteriods (more than 50 have been identified) are ubiquitous in plants, and are present at concentrations of 10 to 100 μg/kg in pollen, 1 to 100 μg/kg in immature seeds, and 10 to 100 ng/kg in shoots and leaves of various plant species (Kripach, et al., 1999). The average daily intake of homobrassinolide via dietary sources is expected to be <10 μg/day. Since the tolerated dose in human adults is estimated to be 600 mg/day, and the average daily intake for homobrassinolide is < 10 μg/day, there is an ample margin of safety.

The homobrassinolide level in plants is about 200 ppb. Assuming the average daily intake of homobrassinolide for humans is 600 mg/kg/day (600 ppm/day), the safety margin can be calculated by dividing 600 ppm by 200 ppb, with the result being 3000-fold safety margin.

Reviewer's Conclusion

The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue. Much of the information submitted is not relevant. The ORNL reviewer for the bacterial mutation assay cited above found it to be unacceptable. Additionally, the ORNL reviewer for the 90-day oral toxicity study in rats found it to be unacceptable. The information submitted is not sufficient to support the requested waiver for teratogenicity.

References Cited

Kripach et al. 1999. In: Brassinosteroids: A New Class of Plant Hormones. Academic Press, New York, NY.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Mammalian Mutagenicity (OPP 152-19)

MRID 47185139

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
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Task Order No. 07-080

Primary Reviewer:
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Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature:
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: FEB 21 2008

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Mammalian Mutagencity Testing (OPP 152-19)
MRID NO:	47185139
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-49
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Mammalian Mutagenicity.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	A test for mammalian mutagenicity is not required, and a waiver is therefore not needed.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Mammalian Mutagenicity (OPP 152-19).

Registrant's Justification

Homobrassinolide in end use products will be applied to crops in very low amounts (parts per million levels). Its solubility in distilled water is 3.18%, and its other physical and chemical properties suggest it possesses no toxic potential to the environment. Under field conditions, homobrassinolide has been found to be rapidly metabolized in plant tissue, and any residues are rapidly degraded or metabolized.

In acute studies, Homobrassinolide Technical was virtually non-toxic (Table 1).

Table 1. Acute toxicity of Homobrassinolide Technical			
Oral LD_{50} (rat)	MRID 47185118	>5000 mg/kg	Toxicity Category IV
Oral LD_{50} (mouse)	MRID 47208903	>5000 mg/kg	Toxicity Category IV
Dermal LD_{50} (rat)	MRID 47185120	>2000 mg/kg	Toxicity Category IV*
Inhalation LC_{50} (rat)	MRID 47185121	2.26 mg/L	Toxicity Category IV
Eye irritation (rabbit)	MRID 47185122	Mild irritant	Toxicity Category III
Skin irritation (rabbit)	MRID 47185123	Not an irritant	Toxicity Category IV
Skin sensitization (guinea pig)	MRID 47185124	Not a sensitizer**	--

*Registrant's classification. ORNL reviewer notes this should be Toxicity Category III

**ORNL reviewer classified this study as unacceptable, but upgradable upon submission of an acceptable positive control study

The following genetic assays using Homobrassinolide Technical were negative: *Salmonella typhimurium* bacterial mutation (4 standard strains tested up to 0.5 µg/plate, with and without metabolic activation) (MRID 47208904); mammalian (mice) chromosomal mutation (up to 2000 mg/kg) (MRID 47208905); and mammalian (mice) DNA damage (up to 2000 mg/kg) (MRID 47185127). These *in vivo* assays are more credible than an *in vitro* mammalian assay.

Homobrassinolide Technical showed minimal toxicity to aquatic organisms. In freshwater bioassays with *Poecilis reticulata*, and *Brachydanio rerio* the 96-hr LC_{50} for Homobrassinolide Technical was 24.56 mg/L and 14.38 mg/L, respectively (MRID 47185129). The 48-hr LC_{50} in *Daphnia magna* was 8.90 mg/L (MRID 47185130).

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. Applying a safety factor of 10 gives an estimated NOAEL of 100 mg/kg/day for a two-year chronic toxicity in rodents. To extrapolate the chronic toxicity NOAEL value of 100 mg/kg/day in rodents to a lifetime exposure value for humans, the 100 mg/kg/day value is multiplied by 60 kg (avg body weight for humans), which would give 6000 mg/day. Dividing the 6000 mg/day value by a safety factor of 10 gives an estimated NOAEL for homobrassinolide exposure in human adults of 600 mg/day. This would be the maximum tolerated dose for human adults. Dividing the adult maximum tolerated dose by a safety factor of 10 gives a maximum tolerated dose of 60 mg/day for special groups, including children.

Homobrassinolide and other brassinosteriods (more than 50 have been identified) are ubiquitous in plants, and are present at concentrations of 10 to 100 μg/kg in pollen, 1 to 100 μg/kg in immature seeds, and 10 to 100 ng/kg in shoots and leaves of various plant species (Kripach, et al., 1999). The average daily human intake of homobrassinolide via dietary sources is expected to be <10 μg/day. Since the tolerated dose in human adults is estimated to be 600 mg/day, and the average daily intake for homobrassinolide is < 10 μg/day, there is an ample margin of safety.

Humans consume plant sterols via food and also as dietary supplements. Since homobrassinolide and other brassinosteroids are biosynthesized from plant sterols (such as campestanol), the consumption of homobrassinolide is considered to be safe.

The homobrassinolide level in plants is about 200 ppb. If the average daily intake of homobrassinolide for humans is 600 mg/kg/day (600 ppm/day), the safety margin can be calculated by dividing 600 ppm by 200 ppb, with the result being 3000-fold safety margin.

Reviewer's Conclusion

Most of the justification submitted by the registrant is irrelevant. A better justification would be that submitted for the genotoxicity waivers (MRIDs 47185132, 47185133 and 47185434), although the ORNL reviewer for the reverse mutation study (MRID 47208904) cited in those waiver requests found it to be unacceptable. Regardless, ORNL does not believe that mammalian mutagenicity testing is required in this instance, and the waiver request is therefore not needed.

References Cited

Kripach et al. 1999. In: Brassinosteroids: A New Class of Plant Hormones. Academic Press, New York, NY.